Evaluating the TEN Test in the Identification and Monitoring of Cochlear Dead Regions and Cochlear De-afferentation in Rats with Noise Induced Hearing Loss

Capstone Document

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Audiology in the Graduate School of The Ohio State University

By

Katherine Anne Kerns

Graduate Program in Audiology

The Ohio State University

2016

Capstone Committee:
Eric C. Bielefeld, Ph.D., Advisor
Gail M. Whitelaw, Ph.D.
Julie Hazelbaker, Ph.D.

Acknowledgements

I gratefully acknowledge my mentor Dr. Bielefeld for guiding me throughout the entire research process. I would also like to thank Dr. Whitelaw and Dr. Hazelbaker for their cooperation throughout the defense process.

Vita

Spring 2011…...……..B.A. in Speech and Hearing Science

2011 to 2012……..Graduate Research Associate, Department of Speech and Hearing Science, The Ohio State University.

2011 to 2013…… Graduate Teaching Associate, Department of Speech and Hearing Science, The Ohio State University.

Fields of Study

Major Field: Audiology

Table of Contents

Chapter 1: Introduction

Cochlear Mechanics

In a normal human auditory system, a sound wave is transmitted as acoustic energy through the outer ear, and transduced into mechanical energy when it travels through the middle ear (Von Bekesy, 1941). The stapes moves in and out of the oval window, which imparts vibration in the fluid that fills the cochlea (Von Bekesy, 1960). These vibrations cause a displacement on the basilar membrane, within the cochlea, at the frequency of the acoustic stimulus. The vibratory wave-motion of the basilar membrane is called a traveling wave. The pressure of the sound is distributed immediately throughout the cochlea, and the traveling wave moves longitudinally toward the helicotrema. The helicotrema is the part of the cochlear labyrinth where the scala tympani and scala vestibuli meet (Von Bekesy, 1970).

The basilar membrane stibuli meet meet where the scala tympani and scala vestibuli meet the frequency of the acoustic stimulus. The vibrator membrane becomes wider as the distance from the stapes to the helicotrema increases, and its stiffness decreases toward the helicotrema (Von Bekesy, 1960). Thus the resonant frequency of vibration of the basilar membrane decreases toward the helicotrema. Larger, flexible membranes have lower resonant frequencies than smaller, stiffer membranes, which have higher resonant frequencies. Therefore, high frequency sounds cause maximum displacement at the base of the basilar membrane and low frequency sounds cause maximum displacement at the apex of the basilar membrane (Von Bekesy, 1960).

With a moderately intense input, such as 50 dB SPL, a particular location along the basilar membrane will be displaced maximally by the characteristic frequency (CF) of

the input signal (Von Bekesy 1960, 1970). It is also true that for that particular location, the basilar membrane will be displaced by frequencies lower than the one that displaces it maximally. In contrast, higher frequencies will displace lower frequency locations only a little, if at all, even at high levels of intensity (Von Bekesy, 1947).

Once a location along the basilar membrane is displaced, the basilar and tectorial membranes pivot about two hinging points, and a shearing force is created (see Figure 1). The stereocilia of the outer hair cells (OHCs), the tallest of which are firmly attached to the tectorial membrane, bend horizontally as a result of the shearing force created by the vertical displacement of the basilar membrane (Dallos, 1996). The stereocilia of inner hair cells (IHCs), which are not attached to the tectorial membrane, are likely sheared by the fluid that is trapped between the stereocilia and tectorial membrane (Slepecky, 1996).

The OHCs exhibit a type of active response in that they change their size in response to auditory stimulation. The shearing of the stereocilia on OHCs triggers a motile change in the size of OHCs. This motility adds energy into the traveling wave and is responsible for the nonlinear movement of the basilar membrane (Ruggero, 1992). This nonlinear movement of the basilar membrane gives the traveling wave additional energy, particularly for low and medium level inputs. As a result of OHC motility, the sensitivity of the cochlea is increased, which makes it possible for IHCs to respond to lower intensity stimuli (Möller, 2000). Thus, OHCs help provide a cochlear amplifier for transducing small vibrations into IHC depolarizations. According to research done by Von Bekesy (1953), the cochlear amplifier of the OHCs appears to increase vibrations along the basilar membrane up to 40 dB.

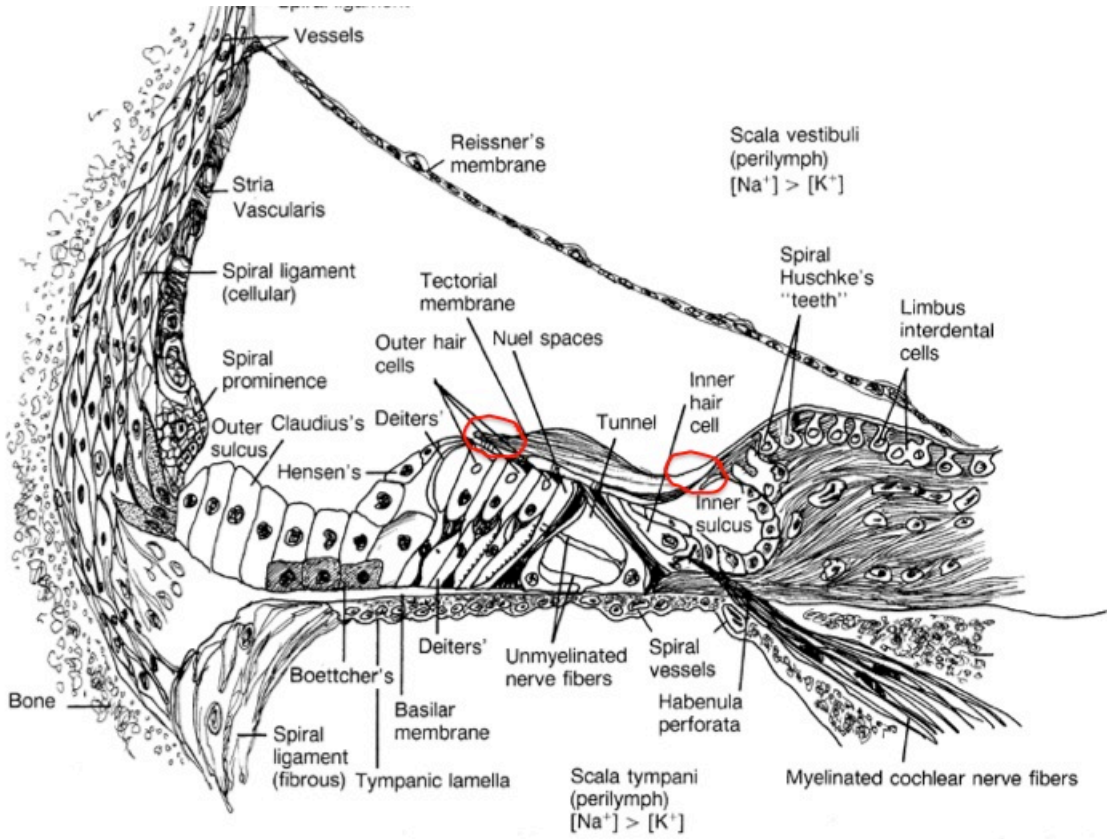

Figure 1: Red circles show the basilar and tectorial membrane pivot about two hinge points which creates a shearing force on OHCs.

Beyond 40 dB, vibrations of the basilar membrane are significant enough to displace the stereocilia of the IHCs and generate an action potential without additional amplification by the OHCs. Since IHCs Sstereocilia do not touch the tectorial membrane, their deflections must be indirect. Thus, either energy from the cochlear amplifier or a deflection of the basilar membrane by a sound greater than 40 dB displaces fluid in the subtectorial space, which shears the stereocilia (Neely & Kim, 1983).

Once the stereocilia of the IHCs are sheared toward the tallest row (in the direction of the limbus) tension in the tip links causes ion channels to open (Hudspeth, 1982). Positive potassium (K+) ions from the endolymph flow through these ion channels and enter the IHCs (Spicer & Schulte, 1996). Depolarization of the negative electrical potentials inside the IHCs induces release of glutamate, a neurotransmitter, into the synapses with afferent auditory nerve fibers. This neurotransmitter is taken up by afferent auditory nerve fibers and the original acoustic signal is transmitted as a neural message up through the central auditory system (Lewis & Hudspeth, 1983).

In addition to the importance of hair cells in contributing energy to the traveling wave (the cochlear amplifier via the OHCs) and in the transduction of the neural signal (via the IHCs), hair cells are also important because they contribute to frequency tuning. It is important to note that the hair cell does not discharge equally to all frequencies/ Different frequencies along the basilar membrane may require more intensity than other frequencies to depolarize the IHC (Kiang & Moxon, 1972). Tuning curves (TCs) are a plot of the intensity needed to make a hair cell (or neurons) fire (above spontaneous rate) across a range of frequencies (Galambos & Davis, 1943).

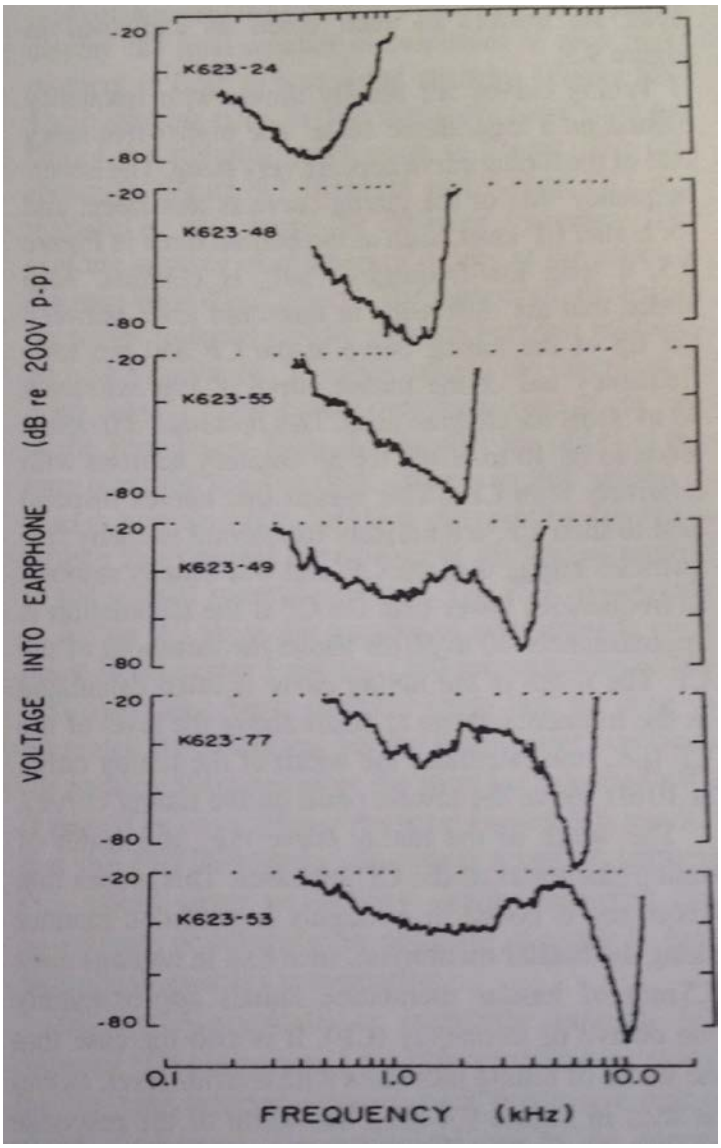

Figure 2: Tuning curves for six single units with different characteristic frequencies. The stimulus level in dB SPL calibrated at the headphones needed to reach each unit's threshold is plotted as a function of stimulus frequency. Note the steep slope on the high-frequency side of the tuning curve and the shallow slope on the low-frequency side. Te three units at the bottom have higher characteristic frequencies and show low frequency "tails" that indicate they are responsive to a wide range of frequencies of stimulation. Adapted from Kiang and Moxon (1972), with permission (Yost, 2007).

Figure 2 shows examples of tuning curves for an individual with normal hearing. Tuning curves are usually drawn with frequency plotted on a logarithmic scale. The higher-frequency side of the tuning curve appears very steep and is referred to as the "tip". The lower-frequency side of the tuning curve is less steep, and for higher CF units a long low-frequency "tail" is obvious. Notice that the difference between the tip of the tuning curve at the CF and the tail of the tuning curve in Figure 2 is about 40-50 dB. This "tip-to-tail" difference tends to be 40-50 dB for all auditory neurons with relatively high CFs. The width of the tuning curve is often calculated as the frequency range 10 dB above the CF and increases as the CF increases. In individuals with a normal auditory system, the tuning curve for a particular CF is sharp. In an undamaged cochlea, when the basilar membrane is displaced at a particular frequency the location of the displacement is precise. This displacement generates a clean frequency response.

It is clear that the IHCs and OHCs play a critical role in the transduction of the acoustic signal in the human auditory system. Unfortunately, in mammals, if the hair cells are severely damaged, they will not recover or be replaced by new hair cells. Thus, the loss or damage of OHCs and/or IHCs leads to permanent hearing impairment.

Hair Cell Damage and Hearing Impairment

OHCs help provide high sensitivity and frequency resolution of the cochlea so that IHCs will transduce sounds across a wide dynamic range and that the auditory system can discriminate small frequency differences in complex sounds. Thus, when OHCs are damaged, there is a significant loss in hearing sensitivity and frequency resolution (Von Bekesy, 1960). Damage to the OHCs causes a loss in hearing sensitivity by impairing the cochlear amplifier (Ruggero, 1992). The loss of the cochlear amplifier

results in a smaller deflection of the basilar membrane for a low intensity sound. This smaller deflection of the basilar membrane manifests as a sensorineural hearing loss on the clinical audiogram. Based on research done by Von Bekesy (1953), one would expect that individuals with severe OHC damage would have a maximum hearing loss of 40 dB. Vibration of the basilar membrane above 40 dB HL is large enough to displace stereocilia of the IHCs without the contribution of the cochlear amplifier (Von Bekesy, 1953).

In addition, OHC damage impairs frequency resolution. OHC damage yields broader tuning along the basilar membrane (see Figure 3). The sensitivity around the tip of an individual's tuning curve is reduced. There appears to be a shift toward higher CFs for the lowest sensitivity of the auditory tuning curve. There is a small change in the low-frequency "tail" of the tuning curve after OHC damage and the "tip-to-tail" sensitivity changes by about 40 dB (Liberman & Dodds, 1984). As a result, all of the frequency selective nonlinear effects of the basilar membrane weaken or disappear (Ruggero, 1992; Yates, 1995; Moore, 2007).

As described previously, the IHCs are transducers for the cochlea. They are responsible for converting the vibration patterns along the basilar membrane into action potentials in the auditory nerve. IHC damage can result in reduced efficiency of transduction of the electro-chemical signal from the organ of Corti to afferent auditory nerve fibers (Moore, 2001).

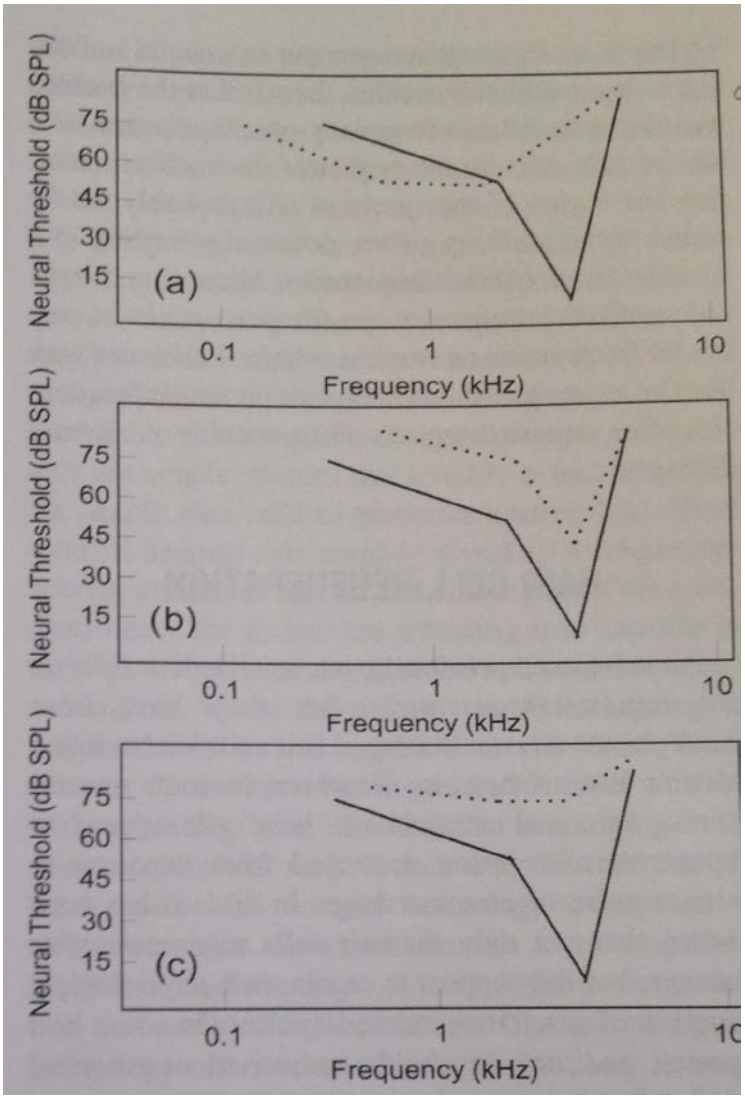

Figure 3: A comparison of a normal auditory nerve tuning curve (solid dark line) with its tip at CF and low frequency tail with tuning curves from animals with severely damaged or missing hair cells (dotted curve) a) Comparison when outer hair cells are severely damaged, showing the loss of the highly tuned tip of the tuning curve, loss of sensitivity, and movement of the tip toward high frequencies. B) comparison when inner hair cells are severely damaged, showing that the tuning curve maintains its overall shape but that there is an overall loss of sensitivity. C) comparison when both inner and outer hair cells are severely damaged, showing a major loss in sensitivity and a significant change in the shape of the tuning curve (Yost, 2007)

IHC damage tends to preserve the shape of the tuning curve but results in a 40 to 50 dB overall loss in the sensitivity of the nerve (see Figure 3). This suggests that damage to the IHCs would result in hearing loss as measured by threshold shift, but since the shape of the tuning curve is not significantly altered, frequency resolution is not affected (Liberman & Dodds, 1984).

When both OHCs and IHCs are damaged, the shape of the tuning curve is altered and there is a very large decrease (75 to 90 dB) in neural sensitivity to the CF location where the damaged OHCs and IHCs are located (see Figure 3). Thus, with damage to both OHCs and IHCs, a more intense signal is required to displace the basilar membrane enough for an individual to detect the sound. A more intense signal is also required to initiate neuronal firing in auditory nerve fibers.

Spiral Ganglion Neuron Damage and Cochlear De-afferentation

In addition to hair cell loss, higher thresholds can also be produced by damage to auditory neurons. Specifically, noise exposure can lead to damage to Type I afferent neurons and their peripheral processes (Puel et al., 1998; Pujol & Puel, 1999; Roberston, 1983; Wang et al., 2002). Afferent auditory nerve fibers swell after noise exposure due to glutamate excitotoxicity (Puel et al., 1998). A large amount of glutamate is released by the IHCs during high levels of noise. These levels of glutamate in the synapses of spiral ganglion neurons (SGNs) can overstimulate the post-synaptic receptor cell bodies, leading to excitotoxicity (Henderson et al., 2006; Le Prell et al., 2007). Toxic concentrations of glutamate trigger entry of fluid into cells, which leads to swelling and can lead to rupturing of cell membranes and degeneration of the cell body (Le Prell et al., 2007). Noise-induced trauma to the dendritic terminals of the SGNs leads to synaptic

uncoupling and loss of function (Pujol & Puel, 1999). After noise- or drug-induced glutamate excitotoxicity, cochlear synaptic ultrastructure and auditory thresholds can recover, suggesting repair or regeneration (Puel, 1995; Puel et al., 1998; Zheng et al., 1997).

After hair cell loss due to noise exposure, SGNs can undergo secondary degeneration, called cochlear de-afferentation, especially in regions with destroyed IHCs (Stankovic et al., 2004; Sugawara, Corfas & Liberman, 2005; Zimmerman et al., 1995). However, primary loss of cochlear fibers does not lead to hair cell or supporting cell loss (Stankovic et al., 2004). The degree of degeneration of SGNs after IHC loss is variable (Sugawara et al., 2005) and can occur within weeks or months after noise exposure (Talaska & Schacht, 2007). Long-term survival of neurons is enhanced by the presence of intact supporting cells (Stankovic et al., 2004; Sugawara et al., 2005). Supporting cells ensheath the unmyelinated portion of the SGNs near the hair cell synapse and express many markers similar to the glial cells in the central nervous system that provide necessary trophic support (Rio et al., 2002; Stankovic et al., 2004). Neurotrophins BDNF and NT-3 can be critical to the survival of SGNs, and are normally released by both hair cells and supporting cells (Stankovic et al., 2004; Sugawara et al., 2007). Interruption of a signaling pathway in the supporting cells that reduces expression of NT-3 leads to massive loss of SGNs without loss of IHCs (Stankovic et al., 2004). The difference in degree of SGN degeneration based on the presence of hair cells and supporting cells is consistent with the dependence of neuronal survival on neurotrophin release (Makary et al., 2011). In regions of IHC loss, degeneration of peripheral axons was first seen at one

week after noise exposure and degeneration of cell bodies was seen eight weeks after noise exposure (Wang et al., 2002).

Since hair cell loss occurs directly after noise exposure and SGN degeneration follows a much longer time course, this degeneration was believed to occur secondarily to hair cell loss (Stankovic et al., 2004; Kujawa & Liberman, 2009). However, recent evidence suggests that primary SGN loss can occur in the absence of hair cell loss over a period of several months to years after noise exposure that induced only temporary threshold shift (TTS) (Kujawa & Liberman, 2006, 2009; Lin et al., 2011). Kujawa and Liberman (2006) exposed subsets of CBA/CaJ mice between the ages of 4-124 weeks to a 100 dB SPL 8-16 kHz octave band noise for two hours while age-matched controls remained unexposed. Animals were then allowed to survive until 2, 16, 32, 64, or 96 weeks after noise exposure in order to examine the relationship between noise exposure and aging. Auditory brainstem response (ABR) thresholds and distortion product otoacoustic emissions (DPOAEs) were recorded before acoustic exposure and before harvest of cochlear tissues at the varying post-exposure intervals. Initial noise-induced threshold shifts were seen in both ABR and DPOAE measures at 2 weeks in young animals while older animals showed no significant threshold shifts. However, at 96 weeks, ABR threshold shifts were seen across all test frequencies while insignificant changes in DPOAEs occurred. These findings suggest that the later threshold shifts were due to IHC and/or auditory nerve pathology and not due to progressive OHC damage. In ears that were exposed and then allowed to age, substantial loss of the SGNs was seen throughout the cochlea without substantial OHC or IHC loss. Since this also occurred in older mice that had only sustained noise-induced TTS, it suggests that neural

degeneration can occur after an exposure that had previously been believed to be fully reversible (Kujawa & Liberman, 2006).

Dead Regions

Sometimes attached afferent auditory nerve fibers and hair cells along the basilar membrane may be completely non-functioning. Places along the basilar membrane with non-functioning OHCs, IHCs, and auditory neurons are referred to as 'dead regions'o(Moore and Glasberg, 1997). A dead region can be characterized as a place in the cochlea that is non-functioning. For example, an area where there are no functioning high frequency hair cells or auditory nerve fibers could be referred to as a basal dead region. An area where there are no functioning low frequency hair cells or auditory nerve fibers could be referred to as an apical dead region. A dead region may be defined by a range of CFs on the basilar membrane where the hair cells are not functioning properly. For example if OHCs and IHCs are non-functional from 7-10 kHz, one would say there is a dead region extending from 7-10 kHz. Neurons at that location of the cochlea will not respond because the OHCs and IHCs are nonfunctioning (Moore et al., 2000).

Properties of Dead Regions: Off-frequency Listening

In cochlear dead regions, stimulation along other regions of the basilar membrane that are intact can still be detected without impairment. In the central nervous system, nonfunctional neurons can re-network and re-wire neuronal pathways to manifest as auditory plasticity. In addition, the properties of basilar membrane displacement, the traveling wave, create conditions of off-frequency listening. A low frequency sound may be detected by neurons tuned to higher frequencies, which can be termed "the upward spread of excitation" (Thornton and Abbas, 1980). This sound detection when the CF on

the basilar membrane is different from that of the tone is known as off-frequency listening (Johnson-Daies & Patternson, 1979).

Off-frequency listening is an important concept in interpreting psychoacoustic results from patients with dead regions (Thornton and Abbas, 1980; Florentine and Houtsma, 1983). Cochlear damage at a given CF may be greater than suggested by the audiometric threshold at that frequency. Due to off-frequency listening, audiometric thresholds at a CF that has a dead region may appear better than profound hearing loss, even though the dead region is not transducing any auditory signals (Gravendeel & Plomp, 1960; Halpin et al., 1994).

From the audiogram, a dead region can be suspected if a) hearing loss is more than 90 dB HL at high frequencies or 75 dB HL at low frequencies b) hearing loss is 40-50 dB at low frequencies with near-normal hearing in the medium and high frequencies c) hearing loss greater than 50 dB HL at low frequencies with somewhat less hearing loss at high frequencies (indicating a low frequency dead region) and d) hearing loss increasing rapidly with increasing frequency (indicating high frequency dead region) (Moore, 2001).

However, standard pure tone audiometric assessment is not sensitive enough to discriminate between OHC damage, IHC damage, strial damage, and SGN damage. Dead regions are therefore not easily diagnosed from the pure tone audiogram because of the downward and upward spread of excitation (Leshowitz, 1977). “True” hearing loss in a dead region should be infinite, but the audiogram sometimes may indicate only a mild or moderate hearing loss (Moore, 2001). The basilar membrane region with CF of X may be incapable of transducing sound, but other basilar membrane regions adjacent to the CF of X are capable of transducing acoustic stimulus X if the intensity of the stimulus is

sufficiently high. This makes diagnosis of dead regions difficult, which is a problem because the presence or absence of dead regions can have serious implications for the fitting of hearing aids (Johansson, 1966). Amplification over a frequency range corresponding to a dead region may not be beneficial. In some cases, in fact, the amplification of sound, particularly in the higher frequencies, actually impairs speech discrimination (Turner & Cummings, 1999). This suggests that it may be beneficial to not amplify frequency components corresponding to a dead region. In addition, it may not be useful to incorporate frequency transposition in hearing aids (McCreery et al., 2012). In light of this information, a test or measure was needed to specifically evaluate the difference between individuals who have hearing loss with functional IHCs and hearing loss with non-functional IHCs (Moore, 2001).

The TEN Test

The Threshold Equalizing Noise (TEN) test is a simple procedure that is indicated for use clinically to detect the presence of one or more hypothetical dead regions and define the limits of the frequency range of a dead region. The test is based on the detection of sine waves in the presence of a broad-band noise, designed to produce almost equal masked thresholds (in dB SPL) over a wide frequency range, for both normal hearing and hearing impaired (without dead regions) listeners (Moore, 2000). For example, a TEN level of 70 dB SPL/ERB_n usually yields a masked threshold of approximately 70 dB SPL in a normal hearing or hearing impaired listener without a dead region. If a pure-tone in a dead region is heard “off-frequency”, the amount of vibration at this remote region will be less than in the dead region, and so the TEN noise will be very effective in masking it. Thus, an abnormally high TEN-masked threshold at an

individual frequency indicates a lack of surviving IHCs, also known as a dead region, at that frequency. Using the example above, a TEN level of 70 dB SPL/ERB_n would yield a masked threshold of approximately 97 dB SPL in an individual with a cochlear dead region (Moore, 2001) due to the inability of the listener to access off-frequency listening because of the presence of the TEN masker.

Lagenbeck (1965) was an early advocate of ocate of ately 97 dB SPL i'. He developed a spectrally shaped masker that would produce equal masked thresholds from 128-8,000 Hz for normal hearing listeners. He reported that if a threshold in noise was much higher than normal, above absolute threshold, then there may be an indication of nerve damage (Moore, 2001). At that time, Lagenbeck did not acknowledge the differences between OHCs and IHCs as well as the effects of the spread of excitation. Now, a threshold equalizing noise is used rather than a spectrally shaped masker to identify nerve damage. The TEN test is, however, comparable to Lagenbeck's genbeckver, comparable to L' because both tests assume that the noise is sufficient to raise the threshold for detecting a signal above absolute threshold to identify cochlear dead regions (Gravendeel & Plomp, 1960).

Individuals with dead regions rely on off-frequency listening (Thornton & Abbas, 1980). The basilar membrane vibration at the remote place where off-frequency listening occurs will generally be less than the amplitude in the dead region. The amount of vibration at this remote region is less than that in the dead region, so the TEN noise is very effective in masking the signal, and will result in shifts in threshold that are far greater than the magnitude of the masker alone would predict. Typically, thresholds will

be higher than 10 dB above the TEN masker level for individuals that have cochlear dead regions (Moore, 2001).

It should be noted that higher thresholds can be produced by central damage (Moore & Glasberg, 1997). Dead regions therefore are not always the cause of higher thresholds (Moore, 2001). Clinicians should incorporate the cross-check principle (Jerger & Hayes, 1976) by using a battery of other measures such as ABR and OAE testing alongside the TEN test to confirm the presence of a dead region.

New Applications of the TEN test

As mentioned above, higher thresholds on the TEN test can be produced by damage to auditory neurons. The degeneration of auditory neurons can occur with damage to the IHC pathway (Stankovic et al., 2004) and in the absence of hair cell loss (Kujawa & Liberman, 2006, 2009). With noise exposure, this degeneration or de-afferentation of auditory nerve fibers becomes more severe gradually, over a period of time. This degeneration can occur weeks or months after noise exposure (Talaska & Schacht, 2007).

When there is de-afferentation of auditory nerve fibers, individuals still have fairly sensitive auditory detection to simple stimuli (Moore, 2007). For example, an individual should still have fairly good detection of pure tones in quiet due to the population of auditory neurons still making synaptic contact with the IHCs. However, individuals with this damage tend to struggle listening to complex stimuli (Vickers, Moore, & Baer, 2001). For example, an individual may struggle when doing a detection task in TEN noise.

The TEN test has traditionally been presented as a test that is "all or nothing". It either confirms or negates the presence of cochlear dead regions (Moore et al., 2000). The current experiment was developed on the premise that the TEN test has been potentially underutilized as a tool for assessing cochlear and neural damage to the auditory system. One goal of the study was to use the TEN test to track the progression of possible cochlear de-afferentation over time after a noise exposure.

Current Study

The current study investigated if the components of ABR with TEN test noise were sensitive measures for the identification and monitoring of cochlear dead regions and cochlear de-afferentation after exposure to hazardous noise. It is assumed that individuals with cochlear dead regions and de-afferentation rely on off-frequency listening which yields better thresholds than that individual's actual cochlear processing at the region of the basilar membrane tuned to that particular frequency. When the TEN noise was used in the current experiment, we expected to see ABR thresholds that more accurately reflect an individual's hearing sensitivity (high thresholds) at that particular frequency. The study utilized absolute and TEN test threshold data. Increases in threshold indicate that the afferent nerve fibers have been damaged in the presence of hair cell pathology (Puel, 1995).

Chapter 2: Methods

Subjects

Twelve Fischer 344/NHsd rats were used in the study. Animals were obtained from Harlan Laboratories at 3-4 months of age and were housed in the Ohio State University Laboratory Animal Resources colony. All procedures involving use and care of the animals were reviewed and approved by The Ohio State University's Institutional Animal Care and Use Committee.

Electrophysiology

In order to assess absolute thresholds and TEN test thresholds, free field ABR was used. For all testing procedures, the rats were anesthetized with inhalant isoflurane (4% for induction, 1.5% for maintenance, 1 L/min oxygen flow rate). Induction was reached in a plastic induction box. After animals were anesthetized, they were moved for testing to a single-walled sound attenuation booth (Industrial Acoustics Company, Bronx, NY) in which anesthesia was delivered through a nose cone. Needle electrodes (Grass Technologies, West Warwick, RI) were placed below both pinnae (non-inverting behind the right ear , inverting behind the left ear) and above the left hind leg (ground). Stimuli were generated using Tucker Davis Technologies (TDT, Gainesville, FL) SigGenRZ version 5.1 software, and were generated in a TDT RZ6 unit. Tone bursts at frequencies of 10 kHz were 1 msec in duration with a 0.5 msec rise/fall time with no plateau. Signals were routed to a speaker (TDT Model MF1) positioned at 90 or 270 degrees azimuth, 3 cm from the ear being tested. The evoked responses were amplified with a gain of 50,000 through use of a headstage (TDT RA4LI) connected to a preamplifier (TDT RA4PA).

Pre-exposure testing

Absolute thresholds were measured in quiet, followed by TEN test thresholds that were measured with TEN noise levels of 30, 50, and 70 dB SPL. The TEN noise used in this study was a narrowband noise one equivalent rectangular bandwidth (ERB) wide at 30, 50, and 70 dB SPL (Moore, 2001). The TEN noise was generated using a real time signal processor (TDT RP2) then delivered to the same TDT MF1 speaker being used to deliver the tone burst stimuli. The TEN noise and tone burst signals were combined to the same speaker with TDT signal mixer.

For absolute and TEN test thresholds, 400 sweeps were averaged at each level of the stimulus using TDT BioSigRZ version 5.1. The stimuli were decreased from 90 dB SPL in 5 dB steps to 5 dB SPL or a level that was at least 10 dB below the lowest level that a response could be detected. Only the right ear was assessed in all subjects. Absolute threshold was defined as the lowest level at which a detectable response could be elicited in quiet. TEN test threshold was defined as the lowest level at which a detectable response could be elicited at TEN levels of 30, 50, and 70 dB SPL.

Noise Exposure

Following baseline ABR recordings, the animals were exposed to noise to induce cochlear injury and potential de-afferentation. Each animal was initially exposed to a 120 dB SPL narrowband noise centered at 10 kHz with a bandwidth of 6 kHz at Day 0. Thresholds were measured post noise exposure at Day 21 and Day 84. Embedded in that noise were 130 dB pSPL impacts, one every second. The noise was created using TDT RPvdsEX version 7.1. The noise was generated using a real time signal processor (TDT RZ6) then amplified by a power amplifier (Marathon DJ-5000, Marathon Professional, New York, NY) and delivered to a speaker. Each animal was anesthetized for the

exposure using the same procedures used for EcochG testing. The speaker was placed 3 inches from the right ear, and the 120 dB SPL level reflects calibration at the level of the right ear. The noise levels were calibrated at the level of the animal's head using a sound level meter (LxT1, Larson Davis Inc., Depew, NY) and a ½" condenser microphone (Model 377B02, PCB Piezotronics, Inc., Depew, NY).

Statistical Analyses

For statistical analyses, the right ears of all subjects were analyzed using a two-factor repeated measures ANOVA (week x intensity of the masker). Due to a significant interaction between week and intensity of the masker, a one-way repeated measures ANOVA and paired sample t-tests were performed. A one-way repeated measures ANOVA was performed to compare the amount of noise exposure at each TEN level. Paired t-test were then performed for the masker levels that had significant main effects to determine which days were different from each other. Right ear threshold data was collapsed across frequency then analyzed using a series of paired t-tests at each time point. A p-value of <0.05 was considered significant in all analyses except paired samples t-tests on which a Bonferroni correction was applied.

Chapter 3: Results

In this study, the independent variables were masking conditions (quiet, 30, 50, and 70 dB SPL of TEN) and day of testing relative to the noise exposure (Pre, Day 21, and Day 84). Results of a two-way repeated measures ANOVA (masking x day) revealed a significant main effect for the masking condition ($F=68.57$, $p<0.01$). A significant main effect was also observed for their day of testing ($F=35.250$, $p<0.01$). Of greatest relevance to the analyses, there was a significant two-way interaction between masking condition and day of noise exposure ($F=6.249$, $p<0.01$). Figure 1 shows mean thresholds (+/- 1 s.d.) for the different TEN masking levels as a function of the test day with respect to the noise exposure.

This two-way interaction indicated that there were significant differences between the different test days depending on the masking condition used. To further assess the significance of the two-way interaction, a series of one-way repeated measures ANOVAs with test day as the variable was assessed for each of the four masking conditions. Results revealed a significant main effects of test day for the quiet ($p<0.0001$), 30 dB ($p<0.0001$), and 50 dB ($p=0.025$) TEN levels. There was no significant difference for thresholds at 70 dB TEN level ($p=0.289$).

To analyze the main effects of test day in the quiet, 30 dB, and 50 dB conditions, paired t-tests were performed to determine which days were different from each other for each masking condition. Paired t-test were first performed for days in the quiet condition. Results revealed that there was a significant difference between pre noise exposure ($M=19.55$, $SD=5.22$) and Day 21 ($M=46.82$, $SD=21.48$) [$t(10)=-4.51$, $p<0.01$]. There was a significant difference between pre noise exposure ($M=19.55$, $SD=5.22$) and Day 84

(M=49.55, SD=14.05) [t(10)=,-8.41, p<0.01]. However, there was not a significant difference between Day 21 (M=46.82, SD=21.48) and Day 84 (M=49.55, SD=14.05) [t(10)=-0.54, p>0.05].

Next, paired t-tests were performed to investigate the 30 dB TEN masking level condition. Results revealed that there was a significant different between pre noise exposure (M=37.73, SD=5.64) and Day 21 (M=55.45, SD=10.60) [t(10)=-5.56, p<0.01]. There was a significant difference between pre noise exposure (M=37.73, SD=5.64) and Day 84 (M=57.27, SD=12.52) [t(10)=-5.75, p<0.01]. However, there was not a significant difference between Day 21 (M=55.45, SD=10.60) and Day 84 (M=57.27, SD=12.52) [t(10)= -0.46, p>0.05].

Finally, paired t-tests were performed to investigate the 50 dB TEN masking level condition. Results revealed that there was a significant difference between pre noise exposure (M=59.09, SD=5.84) and Day 84 (M=68.64, SD=7.45) [t(10)=-3.30,p<0.05]. In addition, there was a significant different between pre noise exposure (M=59.09, SD=5.84) and Day 21 (M=65.91, SD=7.00) [t(10)=-2.10, p>0.05]. However, there was not a significant difference between Day 21 (M=65.91, SD=7.00) and Day 84 (M=68.64, SD=7.45) [t(10)=-0.75, p>0.05].

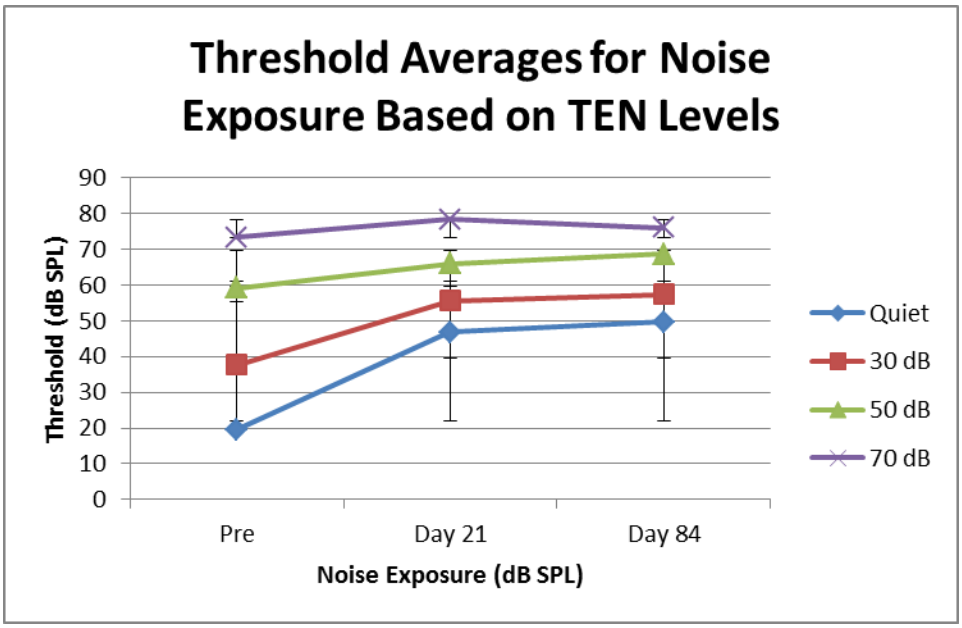

Figure 1: Average threshold data for 12 subjects that were exposed to noise (pre noise exposure, day 21, and day 84) based on TEN levels (quiet, 30 dB, 50 dB, and 70 dB).

To further attempt to understand the findings, the authors decided to assess each animal's responses individually. The 50 dB TEN level was close to the level of the average quiet thresholds at Day 21 (M=46.82) and Day 84 (M=49.55) for Subjects 3, 4, 5, 7, 12, 14, 15, and 16. The 50 dB TEN level therefore provided the closest actual estimate of TEN test results that would be expected in humans for these subjects (with TEN levels typically delivered at 10 dB SL). Table 1 shows the thresholds in quiet pre noise exposure, at Day 21 after noise exposure, and Day 84 after noise exposure. It was expected that some subjects with lower than 45 dB thresholds in quiet at Day 21 or Day 84, (below the red line in Figure 2) would experience a threshold shift to a level greater than 50 dB in the 50 dB TEN masking condition. Such a threshold increase would indicate that the TEN was masking off-frequency listening that was lowering quiet threshold.

Table 1: Thresholds for subjects in quiet.

Thresholds (@10 kHz)	Pre	Day 21	Day 84
3	20	50	50
4	20	45	45
5	25	60	45
7	15	25	40
8	25	75	55
9	25	75	75
11	15	75	65
12	15	25	40
14	10	35	30
15	20	25	35
16	25	25	65
AVG	19.55	46.82	49.55

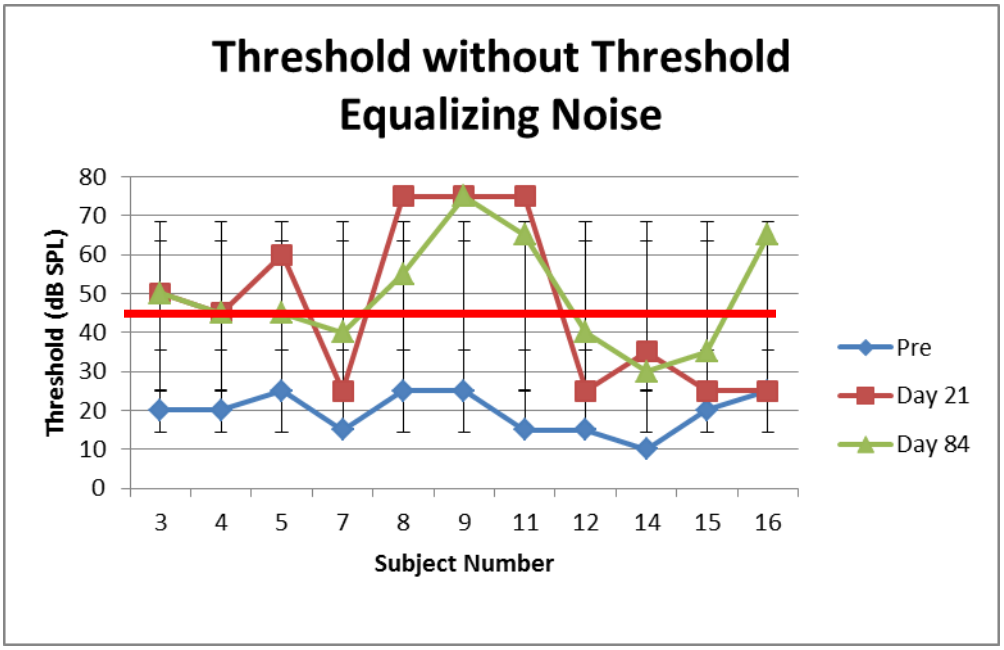

Figure 2: Thresholds for subjects in quiet. Subjects below the red line at 45 dB SPL(subjects 7, 12, 14, and 15) Pre, Day 21, and Day 84 are expected to be affected the most by the 50 dB TEN masker.

Subjects 7, 12, 14, and 15 had thresholds lower than 45 dB in quiet through Day 84. The authors hypothesized that subjects with thresholds less than 45 dB in quiet would have a greater threshold shift with the 50 dB TEN in the post-noise recordings than would have been found in the pre noise condition. At 50 dB TEN (see Table 2 and Figure 3) the authors observed that there was little change in thresholds ($\leq$ 10 dB difference) for Subjects 3, 4, 7, and 12. Subject 5 experienced a 20 dB threshold shift at Day 21, then the subject's threshold went back to the pre noise exposure level at Day 84. The authors observed cumulative increases by 10-30dB in threshold for Subjects 14, 15, and 16. However, it should be noted that Subject 16 had a large threshold shift in quiet at Day 84.

Table 2: Thresholds at 50 dB TEN and threshold shifts for twelve subjects pre noise exposure, at Day 21, and Day 84. Highlighted are the subjects where 50 dB TEN provided adequate masking levels to measure possible cochlear dead regions and cochlear de-afferentation.

Thresholds (@10 kHz)	Pre	Day 21	Day 84	Threshold Shifts	Pre-21	21-84	Pre-84
3	60	70	65		10	-5	5
4	60	70	65		10	-5	5
5	60	80	60		20	-20	0
7	65	55	65		-10	10	0
8	60	65	75		5	10	15
9	65	60	70		-5	10	5
11	65	60	85		-5	25	20
12	60	65	60		5	-5	0
14	55	70	65		15	-5	10
15	55	60	70		5	10	15
16	45	70	75		25	5	30
AVG	59.09	65.91	68.64		6.82	2.73	9.55

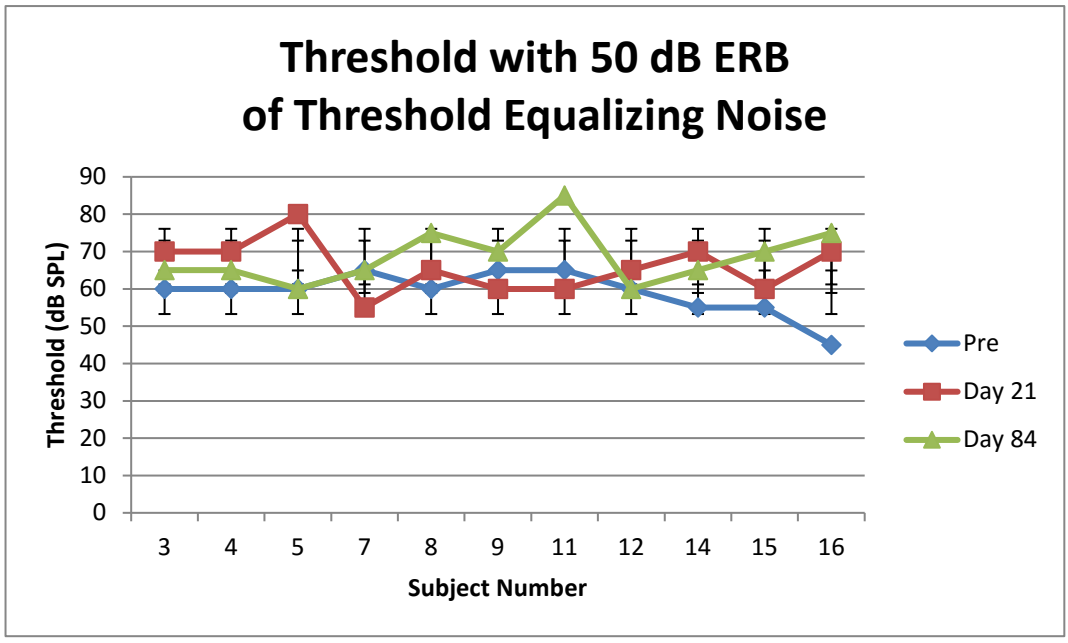

Figure 3: Line graph of thresholds at 50 dB TEN for twelve subjects pre noise exposure, at Day 21, and Day 84.

The 70 dB TEN level was closest to the level of the average thresholds at Day 21 and Day 84 for Subjects 8, 9, and 11. Therefore, the 70 dB TEN level provides the closest actual estimate of human clinical TEN test results for these subjects. Thresholds in quiet increased more for these subjects (see Table 1); therefore, the authors assume that more than damage only to the outer hair cells was done to these subjects to result in the high thresholds in quiet. Table 3 and Figure 4 show the thresholds at 70 dB TEN thresholds pre noise exposure, at Day 21 noise exposure, and Day 84 noise exposure. The authors observed that there was little change in thresholds ($\leq$ 10 dB difference) for Subjects 8 and 9. However, there was a threshold shift greater than 10 dB for Subject 11. The large increases in threshold shift indicated possible cochlear de-afferentation for Subject 11.

Table 3: Thresholds for subjects at 70 dB TEN. Highlighted are the subjects where 70 dB TEN provided adequate masking levels to measure cochlear dead regions and cochlear de-afferentation.

Thresholds (@ 10 kHz)	Pre	Day 21	Day 84	Threshold Shifts	Pre-21	21-84	Pre-84
3	75	85	65		10	-20	-10
4	75	80	75		5	-5	0
5	70	90	70		20	-20	0
7	75	80	90		5	10	15
8	65	70	75		5	5	10
9	70	65	75		-5	10	5
11	75	75	90		0	15	15
12	75	70	65		-5	-5	-10
14	80	80	80		0	0	0
15	70	85	85		15	0	15
16	75	80	65		5	-15	-10
AVG	73.18	78.18	75.91		5	-2.27	2.73

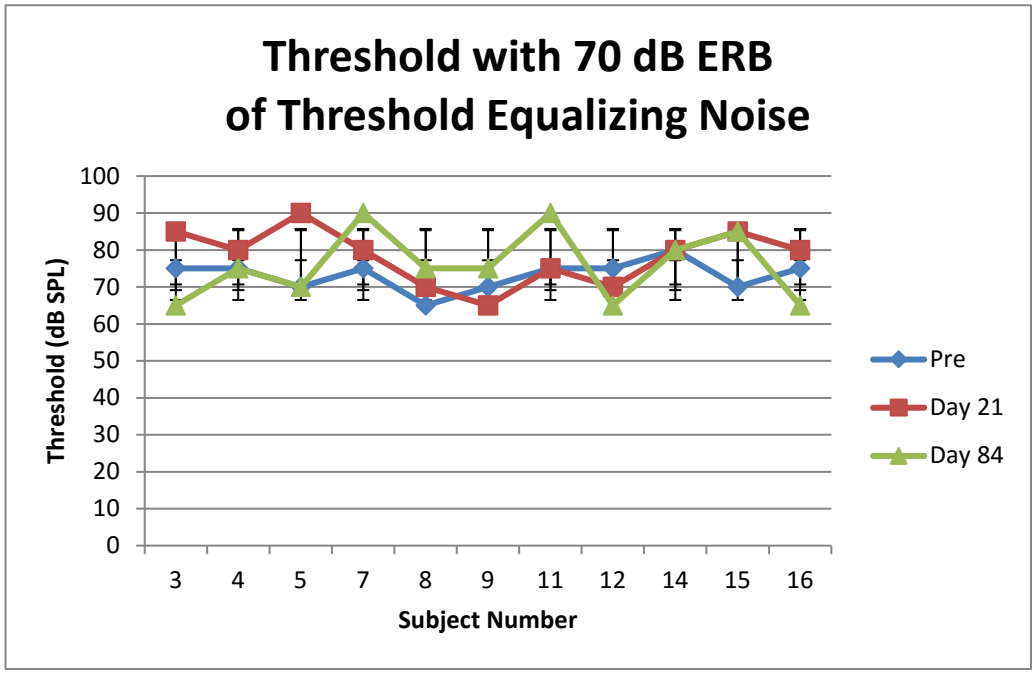

Figure 4: Line graph of thresholds at 70 dB TEN for twelve subjects pre noise exposure, at Day 21, and Day 84

Chapter 4: Discussion

The TEN test has been used as a measure to clinically detect the presence of one or more dead regions, and to define the frequency range of those dead regions. Individuals with dead regions rely on off-frequency listening to detect the presence of sound. The TEN masker in this test is used as a way to limit the involvement of off-frequency listening in detecting the signal to determine an individual's true cochlear performance at a particular frequency region of the basilar membrane. The TEN masking noise is very effective in increasing thresholds that heavily rely on off-frequency listening.

The TEN test has traditionally been used as an "all or nothing" method of measuring damage to the IHCs and OHCs by only identifying the presence or absence of cochlear dead regions. Using the TEN test, one can only say that maximum damage has been done to or that maximum damage has not be done to the hair cells. The genesis for the current study was the general hypothesis that applications of the TEN test have been underutilized. Instead of measuring all-or-nothing differences, the current experiment sought to explore whether it is possible that measured threshold shift from the TEN test could assist in monitoring whether damage is being done directly to the auditory neurons without necessarily affecting the hair cells. Cochlear de-afferentation involves the degeneration of auditory neurons from excessive noise exposure, and occurs over the course of a few weeks or months. The purpose of this study was to investigate the use of the TEN test in identifying cochlear dead regions and cochlear de-afferentation.

The authors of the current study exposed twelve rats to 120 dB SPL noise centered at 10 kHz for 30 minutes. Thresholds were measured pre- noise exposure and then after the noise

exposure at Day 21 and at Day 84. The authors measured ABR thresholds at 10 kHz using 30 dB TEN, 50 dB TEN, and 70 dB TEN. Thresholds were measured pre noise exposure, at Day 21 post noise exposure, and at Day 84 post noise exposure. A two-factor repeated measures ANOVA (week x intensity of the masker), a one-way repeated measures ANOVA, paired sample t-tests, and descriptive statistics were used in an attempt to identify the possible presence of cochlear dead regions or cochlear de-afferentation.

In quiet, the average threshold shift was only approximately 30 dB. Based on how little threshold shift there was, one can infer that dead regions were not successfully created during this experiment. Research indicates that dead regions typically occur when thresholds on the audiogram are around 90 dB at high frequencies and 75-80 dB at low frequencies (Moore, 2001). In this experiment the average threshold at Day 84 was only 50 dB SPL. The 30 dB threshold shifts were a good damage level for assessing cochlear de-afferentation, as there is a significant degree of cochlear damage evident, but still enough room supra-threshold to make physiologic recordings. Noise that induces a small amount of temporary threshold shift is not associated with de-afferentation.

Using the 30 dB masker, there was a large difference between thresholds in quiet and masked thresholds pre noise exposure, $p<0.001$. However, after the rats were exposed to noise, the average thresholds exceeded the level of the masker (M=46 dB). There was, therefore, very little difference between thresholds in quiet and thresholds using the 30 dB masker after the rats were exposed to noise, nor was a difference anticipated. The 30 dB masker undermasked thresholds post noise exposure, which is why there was very little difference between thresholds in quiet and thresholds at 30 dB TEN starting at Day 21.

Using the 50 dB masker provided the best estimate of TEN test thresholds that would be used in humans (10 dB SL) for Subjects 3, 4, 5, 7, 12, 14, 15, and 16. At 50 dB subjects were not undermasked or overmasked. Differences in thresholds at 50 dB TEN were noted between pre and post conditions but not between post noise exposure conditions. However, there was not a huge jump in thresholds pre noise (M=59.1 dB) exposure and post noise exposure at Day 21 (M=65.9 dB) and Day 84 (M=68.6 dB). The presence of a true cochlear dead region would increase reliance of the auditory system on off-frequency listening. If the subjects were relying on off-frequency listening, one would have expected to see huge threshold shifts after rats had been exposed to noise at 50 dB TEN. The average threshold shift between pre and post noise conditions at 50 dB TEN was not large. The average threshold shift between pre noise exposure thresholds and threshold at Day 21 was 6.8 dB. The average threshold shift between pre noise exposure thresholds and threshold at Day 84 was 9.5. One can therefore infer that dead regions were not created as a result of the noise exposure administered during this experiment, but the possibility of cochlear de-afferentation could account for the post-noise shifts in the 50 dB TEN condition.

Further observations of threshold shifts for individual subjects at 50 dB TEN provided insight into the presence of cochlear de-afferentation as a result of noise exposure, measured by the TEN test. The authors observed that there was little change in thresholds ($\leq$ 10 dB difference) for Subjects 3, 4, 7, and 12. Subject 5 experienced a 20 dB threshold shift at Day 21, then the threshold went back to pre noise exposure levels at Day 84. There may have been a temporary threshold shift of Day 21 and the subject recovered from this threshold shift by Day 84. Perhaps in response to recovering from the temporary threshold shift, Subject 5's efferent

auditory system generated a strong inhibitory protective response to noise exposure on Day 84, which did not cause a permanent threshold shift after noise exposure.

At 50 dB TEN, the authors observed cumulative increases by 10-30 dB in threshold for Subjects 14, 15, and 16. However it should be noted that Subject 16 had a large threshold shift in quiet. The authors hypothesized that the large threshold shift observed for Subject 16 was not a result of the masker. Rather, the large threshold shift for Subject 16 observed at 50 dB TEN was likely due to the threshold shift observed in quiet. The authors therefore concluded that the threshold shifts observed for Subjects 14 and 15 using the 50 dB TEN masker were indicators of possible cochlear de-afferentation using the TEN test.

At 70 dB TEN, the masker shifted thresholds the same amount pre noise exposure and after rats were exposed to noise. The reason there was little change in the thresholds for the majority of the subjects is because thresholds pre and post noise exposure did not exceed 70 dB. The 70 dB masker provided too much masking to rats before rats were exposed to noise and after rats had been exposed to noise. The 70 dB masker overmasked thresholds pre and post noise exposure, which is why there was very little difference between thresholds in quiet and thresholds at 70 dB TEN throughout this experiment. Three subjects did have large threshold shifts in quiet from the noise exposure. These subjects were hypothesized to have greater OHC damage than the other subjects in the study. 70 dB TEN was therefore the ideal masker to use for subjects 8, 9, and 11 because they had very large threshold shifts in quiet.

The 70 dB TEN level was closest to the level of the average thresholds at post-noise exposure (M=73.18) Day 21 (M=78.18) and Day 84 (M=75.90) for Subjects 8, 9, and 11. This TEN level provides the closest actual estimate of TEN test results we would expect in humans (with TEN levels approximately 10 dB SL). Figure 5 shows the thresholds at 70 dB TEN

thresholds pre noise exposure, at Day 21 noise exposure, and Day 84 noise exposure. The authors observed that there was little change in thresholds ($\leq$ 10 dB difference) for Subjects 8 and 9. However, there was a threshold shift greater than 10 dB for Subject 11. This threshold shift suggests the possibility that Subject 11 experienced cochlear de-afferentation as a result of noise exposure. It is reasonable to assume that because a 70 dB masker was more appropriate to use than a 50 dB masker on this subject, that Subjects 8, 9, and 11 may also have more outer hair cell damage than the other subjects in this study. Therefore, Subject 11 experienced cochlear de-afferentation as well as more OHC damage compared to Subjects 14 and 15 who only experienced cochlear de-afferentation.

In summary, Subjects 11, 14, and 15 demonstrated evidence of possible cochlear de-afferentation (>10 dB of threshold shift) as indicated by the TEN test. It should be noted that Subject 11 had increased thresholds in quiet compared to Subjects 14 and 15, and therefore likely had more OHC loss. The authors are not confirming the presence of cochlear de-afferentation. Rather, the authors of this study are simply hypothesizing that it is a possible underlying pathology based on the results of this study.

Limitations

This study was limited by the parameters of the experiment. Using fixed masker levels at 30 dB, 50 dB, and 70 dB TEN did not provide accurate TEN masking levels for all subjects. According to the TEN test, masking should be performed at 10 dB SL. Using 30 dB TEN undermasked subjects and did not prove to be useful in this experiment. While 50 dB and 70 dB TEN were useful in assessing the presence of cochlear de-affereention and cochlear dead regions, not all subjects were assessed at 10 dB SL. Since all subjects were not assessed at 10 dB SL, limited conclusions can be drawn from these data relative to the clinical TEN test. The fixed

masker levels were chosen because this approach had never been attempted before, and this project represents an initial exploration into the TEN test in noise-exposed rats. Follow-up studies would use a flexible 10, 20, or 30 dB SL masking condition instead of the fixed 30, 50, and 70 dB SPL maskers.

The frequency that was used to assess the presence of cochlear de-afferentation and cochlear dead regions was 10,000 Hz. Using a rat model, this would have simulated the effects of creating a low frequency dead region in a human (if the noise exposure had been severe enough to create cochlear dead regions). It should be noted that low frequency dead regions are uncommon. Low frequency dead regions that are created as a result of noise exposure are even more uncommon. It is more likely that a high frequency dead region would result from excessive noise exposure.

In addition, testing only one frequency provides a limited picture of what is happening in the mammalian cochlea as a result of noise exposure. Noise exposure centered around a particular frequency can cause widespread damage, that affects surrounding frequencies/hair cells along the basilar membrane in the cochlea. However, testing multiple frequencies would provide more information about damage to the hair cells and auditory neurons along the basilar membrane. Future studies should test other frequencies to get a clearer picture of how noise exposure affects areas throughout the cochlea.

Also, this study utilized ABR rather than behavioral puretones to administer the TEN test. Most applications of the TEN test have involved behavioral assessment via puretone testing. Theoretically, ABR and puretone testing are both effective ways to acquire information about thresholds of hearing. However, there is limited information to suggest that ABR is a valid test to pair with the TEN test. Using ABR to administer the TEN test may have been a significant

factor that affected the results of this study. An untested aspect of the study was the use of TEN in combination with ABR amplitude data. Previous studies of cochlear de-afferentation (Kujawa and Liberman, 2009; Lin et al., 2011) demonstrated that cochlear de-afferentation induces reductions in ABR amplitude input-output functions. Assessing ABR amplitudes in TEN masking conditions may represent a sensitive test of de-afferentation that is yet to be explored.

Finally, future studies should consider observing mammalian cochlea's post-mortem after noise exposure and performing the TEN test. Cochlear histopathology extended beyond the scope of the current study, but is a relevant examination for this type of experiment in the future. Thus far, outcomes of the TEN test on diagnosing cochlear dead regions are theoretical. Tissue histology of the mammalian cochleae would need to be completed to prove the actual existence of cochlear de-afferentation or cochlear dead regions. If the authors had looked at the hair cells in post-mortem mammalian cochleae, more concrete conclusions about cochlear damage could be made. However, the authors are limited in making conclusions about this study because mammalian cochleae were not observed post noise exposure.

Future Studies

Future research to expand on this study should keep the following in mind. First, future studies should administer TEN masking levels 10 dB above thresholds in quiet. Administering threshold at 10 dB SL will provide more accurate information about threshold shifts in subjects being assessed. Second, future studies should seek to simulate the effects of creating a high frequency dead region by testing higher frequencies using the TEN test. Dead regions as a result of noise exposure are often observed in the high frequencies. In addition, testing more than one frequency may provide a fuller picture of what is happening in the cochlea as a result of noise exposure. Further, studies of ABR amplitude input-output functions may increase the viability of

this testing approach. Finally, future studies should examine cochleae post-mortem to draw definitive conclusions about the presence or absence of cochlear de-afferentation and/or cochlear dead regions.

Conclusions

In summary, at 50 dB and 70 dB TEN, which provided the best estimate of accurate TEN test thresholds for subjects in this study, there was not a large enough increases in thresholds to indicate the presence of cochlear dead regions ($\leq$ 30 dB of threshold shift). However, threshold shifts at 50 dB TEN for Subjects 14 and 15 and thresholds shifts at 70 dB TEN for Subject 11 indicated the presence of cochlear de-afferentation (> 10 dB of threshold shift). More research is needed to determine if the TEN test is an effective test in identifying cochlear dead regions and if further applications of the TEN test, in monitoring cochlear de-afferentation, are plausible.

References

Dallos, P. (1996). Overview: cochlear neurobiology. In *The cochlea* (pp. 1-43). Springer New York.

Florentine, M., & Houtsma, A. J. (1983). Tuning curves and pitch matches in a listener with a unilateral, low‐frequency hearing loss. *The Journal of the Acoustical Society of America*, *73*(3), 961-965.

Galambos, R., & Davis, H. (1943). The response of single auditory-nerve fibers to acoustic stimulation. *Journal of Neurophysiology*, *6*(1), 39-57.

Gravendeel, D. W., Plomp, R., & Eng, M. (1960). Perceptive bass deafness. *Acta oto-laryngologica*, *51*(5-6), 548-560.

Halpin, C., Thornton, A., & Hasso, M. (1994). Low-frequency sensorineural loss: clinical evaluation and implications for hearing aid fitting. *Ear and hearing*, *15*(1), 71-81.

Henderson, D., Bielefeld, E.C., Harris, K.C., & Hu, B.H. (2006). Role of Oxidative Stress in Noise-Induced Hearing Loss. *Ear & Hearing*, *27*(1). 1-19.

Hudspeth, A. J. (1982). Extracellular current flow and the site of transduction by vertebrate hair cells. *The Journal of Neuroscience*, *2*(1), 1-10.

Hudspeth, A. J. (1989). How the ear's works work. *Nature*, *341*(6241), 397-404.

Jerger, J. F., & Hayes, D. (1976). The cross-check principle in pediatric audiometry. *Archives of otolaryngology*, *102*(10), 614-620.

Johansson, B. (1966). The use of the transposer for the management of the deaf child. *International Journal of Audiology*, *5*(3), 362-372.

Johnson‐Davies, D., & Patterson, R. D. (1979). Psychophysical tuning curves: Restricting the listening band to the signal region. *The Journal of the Acoustical Society of America*, *65*(3), 765-770.

Kiang, N. S., & Moxon, E. C. (1972). Physiological considerations in artificial stimulation of the inner ear. *The Annals of otology, rhinology, and laryngology*, *81*(5), 714.

Kujawa, S.G. & Liberman, M.C. (2006). Acceleration of Age-Related Hearing Loss by Early Noise Exposure: Evidence of a Misspent Youth. *The Journal of Neuroscience*, *26*(7). 2115-2123.

Kujawa, S.G. & Liberman, M.C. (2009). Adding Insult to Injury: Cochlear Nerve Degeneration after “Temporary” Noise-Induced Hearing Loss. *The Journal or Neuroscience, 29*(45). 14077-14085.

Langenbeck, B. (1965). *Textbook of practical audiometry*. Williams & Wilkins Company.

Le Prell, C.G., Yamashita, D., Minami, S.B., Yamasoba, T. & Miller, J.M. (2007). Mechanisms of Noise-Induced Hearing Loss Indicate Multiple Methods of Prevention. *Hearing Research, 226*(1-2). 22-43.

Leshowitz, B. (1977). Speech intelligibility in noise for listeners with sensorineural hearing damage. *IPO annual progress report*, *12*(1), 1-25.

Lewis, R. S., & Hudspeth, A. J. (1983). Voltage-and ion-dependent conductances in solitary vertebrate hair cells.

Liberman, M. C., & Dodds, L. W. (1984). Single-neuron labeling and chronic cochlear pathology. III. Stereocilia damage and alterations of threshold tuning curves. *Hearing research*, *16*(1), 55-74.

Lin, H.W., Furman, A.C., Kujawa, S. & Liberman, M.C. (2011). Primary Neural Degernations in the Guinea Pig Cochlea After Reversible Noise-Induced Threshold Shift. *Joural of the Association for Research in Otolaryngology, 12*. 605-616.

Makary, C.A., Shin, J., Kujawa, S.G., Liberman, M.C. & Merchant, S.N. (2011). Age-Related Primary Cochlear Neuronal Degeneration in Human Temporal Bones. *Journal of the Association for Research in Otolaryngology*, *12*. 711-717.

McCreery, R. W., Venediktov, R. A., Coleman, J. J., & Leech, H. M. (2012). An evidence-based systematic review of frequency lowering in hearing aids for school-age children with hearing loss. *American journal of audiology*, *21*(2), 313-328.

Møller, A. R. (2000). *Hearing: its physiology and pathophysiology*. Academic Press.

Moore, B. C. (2001). Dead regions in the cochlea: Diagnosis, perceptual consequences, and implications for the fitting of hearing aids. *Trends in Amplification*, *5*(1), 1-34.

Moore, B. C. (2007). *Cochlear hearing loss: physiological, psychological and technical issues*. John Wiley & Sons.

Moore, B. C. J., & Glasberg, B. R. (1997). A model of loudness perception applied to cochlear hearing loss. *Auditory Neuroscience*, *3*(3), 289-311.

Moore, B. C. J., Huss, M., Vickers, D. A., Glasberg, B. R., & Alcántara, J. I. (2000). A test for the diagnosis of dead regions in the cochlea. *British journal of audiology*, *34*(4), 205-224.

Musiek, F. E., & Baran, J. A. (2007). *The auditory system: anatomy, physiology and clinical correlates*. Allyn & Bacon.

Neely, S. T., & Kim, D. O. (1986). A model for active elements in cochlear biomechanics. *The journal of the acoustical society of America*, *79*(5), 1472-1480.

Pickles, J. O. (1988). *An introduction to the physiology of hearing* (Vol. 2). London: Academic press.

Puel, J.L. (1995). Chemical synaptic transmission in the cochlea. *Progress in Neurobiology, 47*. 449-476.

Puel, J.L., Ruel, K., d'Aldin, C.G. & Pujol, R. (1998). Excitotoxicity and repair of cochlear synapses after noise trauma. *NeuroReport, 9*. 2109-2114.

Pujol, R. & Puel, J.L. (1999). Excitotoxicity, Synaptic Repair, and Functional Recovery in the Mammalian Cochlea: A Review of Recent Findings. *Annals of the New York Academy of Science, 884.* 249-254.

Rio, C., Dikker, P., Liberman, M.C., & Corfas, G. (2002). Glial fibriallary acidic protein expression and promoter activity in the inner ear of developing and adult mice. *Journal of Comparative Neurology*, *442*. 156-162.

Robertson, D. (1983). Functional significance of dendritic swelling after loud sounds in the guinea-pig cochlea. *Hearing Research, 9.* 263–278.

Ruggero, M. A. (1992). Responses to sound of the basilar membrane of the mammalian cochlea. *Current opinion in neurobiology*, *2*(4), 449-456.

Slepecky, N. B. (1996). Structure of the mammalian cochlea. In *The cochlea* (pp. 44-129). Springer New York.

Spicer, S. S., & Schulte, B. A. (1996). The fine structure of spiral ligament cells relates to ion return to the stria and varies with place-frequency. *Hearing research*, *100*(1), 80-100.

Stankovic, K., Rio, C., Xia,A., Sugawara,M., Adams,J.C.,Liberman, M.C., Corfas, G. (2004) Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. *Journal of Neuroscience, 24*. 8651–8661.

Sugawara, M., Corfas, G. & Liberman, M.C. (2005). Influence of Supporting Cells on Neuronal Degeneration After Hair Cell Loss. *Journal of the Association for Research in Otolaryngology, 6*(2). 136-147.

Sugawara, M., Murtie, J.C., Stankovic, K.M., Liberman, M.C., & Corfas, G. (2007). Dyanmic Patterns of Neurotrophin 3 Expresion in the Postnatal Mouse Inner Ear. *The Journal of Comparitive Neurology, 501*. 30-37.

Talaska, A.E. & Schacht, J. (2007). Mechanisms of noise damage to the cochlea. *Audiological Medicine, 5*. 3-9.

Thornton, A. R., & Abbas, P. J. (1980). Low-frequency hearing loss: Perception of filtered speech, psychophysical tuning curves, and masking. *The Journal of the Acoustical Society of America*, *67*(2), 638-643.

Turner, C. W., & Cummings, K. J. (1999). Speech audibility for listeners with high-frequency hearing loss. *American Journal of Audiology*, *8*(1), 47-56.

Vickers, D. A., Moore, B. C., & Baer, T. (2001). Effects of low-pass filtering on the intelligibility of speech in quiet for people with and without dead regions at high frequencies. *The Journal of the Acoustical Society of America*, *110*(2), 1164-1175.

Von Békesy, G. (1941). On the measurement of the amplitude of vibration of the ossicles with a capacitive probe. *Akustishe Zeitschrift*, *6*(1), 16.

Von Békésy, G. (1947). The variation of phase along the basilar membrane with sinusoidal vibrations. *The Journal of the Acoustical Society of America*, *19*(3), 452-460

Von Békésy, G. (1953). Description of some mechanical properties of the organ of Corti. *The Journal of the Acoustical Society of America*, *25*(4), 770-785.

Von Békésy, G. (1960). *Experiments in hearing* (Vol. 8). E. G. Wever (Ed.). New York: McGraw-Hill.

Von Békésy, G. (1970). Travellng waves as frequency analysers in the cochlea. *Nature, 225*, 1207-1209.

Wang, Y., Hirose, K. & Liberman, M.C. (2002). Dynamics of Noise-Induced Cellular Injury and Repair in the Mouse Cochlea. *Journal of the Association for Research in Otolayrngology, 3*. 248-268.

Yates, G. K. (1995). Cochlear structure and function. *Hearing*, 41-74.

Yost, W. A., & Schlauch, R. S. (2007). Fundamentals of hearing: an introduction. *The Journal of the Acoustical Society of America*, *110*(4), 1713-1714.

Zheng, X.Y., Henderson, D., Hu, B.H., & McFadden, S.L. (1997). Recovery of structure and function of inner ear afferent synapses following kainic acid excitotoxicity. *Hearing Research, 105*. 65-76.

Zimmerman, M.,, Picciotto, M.R., Ferrari, R., Cocchi, D. & Changeux, J.P. (1999). Increased neurodegeneration during aging in mice lacking high-affinity nicotine receptors. *EMBO Journal, 18*. 1235-1244.